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## **Effect of Cannabis Inhalation on the Hippocampus of Male Wistar Rats**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Cannabis is the second most commonly smoked substance after tobacco, with an estimated 160 million users (3.8% of the world's population of 15-64 year olds). There are other smokeless forms of cannabis consumption like being added to food. This study is focused on the effect of cannabis inhalation on the hippocampus of Wistar rats. Twenty (20) male Wistar rats weighing 70-100g were separated into 4 groups of 5 rats each; Group A served as positive control and received water and rat chow. Group B rats were placed in the ventilation box without exposure to the cannabis smoke for 10 minutes daily throughout the duration of this study. Group C were exposed to 1g of Cannabis smoke for 10-minutes daily for 14 days while Group D were exposed to 2g of Cannabis smoke for 15-minutes. 24 hours after the last exposure, rats were weighed and subjected to Morris water maze neurobehavioural test for memory. The rats were then sacrificed by cervical dislocation and the brain was harvested. Some were homogenized and centrifuged for biochemical assay while the others were fixed in 10% formal saline for histological tissue processing using the H & E. The findings of this study reveal that cannabis smoke does not affect Wistar rats' body weight and brain weight on short-term exposure. However, it shows mild focal inflammations and alterations in the normal neuronal architecture of the hippocampal cells. On the other hand, the study revealed a decreased learning pattern and memory retrieval time following short-term cannabis smoke exposure in neurobehavioural parameters.

**Keywords:** Cannabis; hippocampus; memory; Morris water maze.

## 1. INTRODUCTION

“Cannabis specifically refers to the green, brown, or gray mixture of dried, shredded leaves, stems, seeds, and flowers of the cannabis plant; it’s called Marijuana” [1]. “It is the second most commonly smoked substance after tobacco, with an estimated 160 million users (3.8% of the world’s population of 15-64 year olds)” [1]. “Cannabis exists in three forms: herbal cannabis, the dried leaves, and flowering tops. Cannabis is used to relieve neuropathic and chronic pain. It contains diverse phytochemicals of biological activities and therapeutic influence, such as cannabinoids, terpenes, and phenolic compounds. Cannabinoids are the most therapeutic component, because of its wide range of pharmaceutical effects in humans, including psychotropic activities” [2]. “The raw plant material contains essential fatty acids, essential amino acids, dietary fiber, enzymes, vitamins, minerals, flavonoids, carotenoids, terpenes, and phytocannabinoid acids. Raw cannabis leaves, stems, stalks, and seeds can provide the body with almost all of the essential nutrients including carbohydrates, protein, fat, water, vitamins, minerals, trace amounts of calcium, sodium, potassium, and omega-3 fatty acids” [3]. Audu [4] reported “presence of alkaloids, flavonoids, cardiac glycosides, resins, terpenes and steroids while the proximate composition had elevated levels of 6.87% moisture, 23% crude protein, 19.97% lipid and 11.8% Ash and 18.95% fibre. Crude fibre of 70% in the stem and 25.36% crude fiber in the seeds”.

*C. sativa* leaf contains 9 essential amino acids (EAA), which have good concentration of methionine and lysine. Oladimeji and Valan [5] reported “the presence of Alkaloid, flavonoids, cardiac glycosides, terpenes & steroids, and resins”. “Also, present were Cannabigerol (CBG), Cannabichromene (CBC), Cannabidiol (CBD), 9-Tetrahydrocannabinol (THC), 8-THC, Cannabicyclol (CBL), Cannabielsoin (CBE), Cannabinol (CBN) and Cannabinodiol (CBND) , Cannabitriol (CBT)” [6]. “The most common route of consumption of cannabis is smoking as with cigarette” [1]. “In contrast, others use it as an ingredient in foods, and are also taken in beer. Two cannabinoid receptor systems; cannabinoid 1 receptor (CB1R) and cannabinoid 2 receptor (CB2R) are present in the brain. The CB1Rs are located in the substantia nigra, basal ganglia, limbic system, hippocampus, and cerebellum”

[7,8]. “Also, they are expressed in the visceral organs like the liver, thyroid, uterus, bones and testicular tissue” [1]. “The CB2Rs are expressed significantly in immune cells, spleen and the gastrointestinal system, but scantily in the brain and peripheral nervous system” [9,10].

A report revealed that Cannabis exposure result in impairments of executive function, including reversal learning, set shifting, and working memory [11]. Also, findings from Broyd et al., [12] and Cohen et al., [13] revealed that “impairment of executive function in synthetic cannabinoid users compared with recreational users of cannabis and non-users”. Yucel et al., [14] reported a decrease in hippocampal volumes following cannabis. Report from Beale et al., [15] reported “restorative effect of CBD on the subicular and CA1 subfields in current cannabis users, especially those with greater lifetime exposure to cannabis”. Owolabi et al., [16] reported that cannabis exposure results in altered individual neurons morphologies and the spatial distribution of the cells in the Cornu Ammonis and dentate gyrus at higher concentration. Despite, numerous studies on cannabis on cognitive and hippocampus architectures, there are limited study on the effect of cannabis on the hippocampus on animal model in this research sphere.

Obonga et al., [17] reported “the formulation of *C. sativa* resin as a syrup using lipophilic and hydrophilic carriers improves significantly the anti-inflammatory activity of Cannabis. Thus, syrup is an efficient and alternative vehicle to the traditional smoking for oral delivery of *C. sativa* as an anti-inflammatory agent”. Akinola et al., [18] reported “the neurobehavioural effects of daily oral ingestion of *C. sativa* and its modulatory changes in oxidative stress parameters in mice brain tissues. Neurobehavioural activities were assessed by observing animals rearing, grooming, ambulation, head dipping and freezing times. The animals fed with cannabis-diet displayed significantly reduced anxiety but statistically insignificant locomotory function, exploratory tendencies and neophilia, in a quantity dependent manner relative to the controls. Cannabis demonstrated both antioxidant and oxidative stress tendencies”. Kim et al., [19] reported that “regular cannabis use can alter brain function, especially in networks that support working memory, attention, and cognitive control

processing. Also, regular cannabis use can alter brain function, especially in networks that support working memory, attention, and cognitive control processing. The hippocampus and caudate nuclei specifically showed aberrant structural and functional coupling. These structures have high CB1 receptor density and may also be associated with changes in learning and habit formation that occur with chronic cannabis use”.

## 2. METHODOLOGY

### 2.1 Location and Duration of the Study

This study was carried out in the Animal House, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. The rats were acclimatized for a period of two weeks after which the test substances were administered for two weeks. The entire experiment lasted for four weeks

### 2.2 Procurement of *Cannabis sativa*

Dried leaves of *Cannabis sativa* was obtained from locals in Otolu Nnewi Anambra State.

### 2.3 Experimental Animals

Twenty (20) adolescent female Wistar rats of about 3 months old weighing 80-120g were obtained from the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Animals were kept in standard cages at room temperature of  $27\pm 2^{\circ}\text{C}$ . The animals were maintained with normal laboratory chow (Grower feed) and water *ad libitum*. The animals were acclimatized for two weeks before exposure to cannabis smoke.

### 2.4 Experimental Design

Group A served as Positive Control and received only distilled water and rat chow. Group B were placed in the ventilation box without exposure to the cannabis smoke for 10 minutes daily throughout the duration of this study. Group C were exposed to a wrap of 1g of Cannabis smoked for 10-minutes daily for 14 days. Group D were exposed to 2g of Cannabis smoke for 15-minutes. Cannabis exposure was done daily between 6 am and 8 am and the experiment lasted for two weeks.

### 2.5 Neurobehavioural Procedure

The Neurobehavioural test was done using Morris water maze (MWM) as described by Barnhart et al. [20]. The test was conducted in a round water pool 65 cm in diameter and 45 cm deep. The pool was filled to a depth of 30 cm with water made opaque with non-fatty milk. The escape platform was a 25-cm<sup>2</sup> block, placed in the center of the pool submerged 1 cm beneath the water surface. The platform remained in the same position throughout the learning training phase and trials and was removed from the pool during the test proper.

### 2.6 Termination and Sample Collection

Twenty four hours after the last exposure, the rats were weighed and subjected to Morris water maze neurobehavioural studies for memory. After then, the animals were sacrificed by cervical dislocation and the brains excised. Some of the brains were homogenized and centrifuged for biochemical studies. The other brains were fixed in 10% formal saline for histological studies using H&E. Tissue sections were produced by normal histological methods of fixation, dehydration, clearing, impregnation, embedding, sectioning, mounting, and staining.

### 2.7 Statistical Analysis

Data was analysed using Statistical Package for Social Sciences (SPSS Version 25). The results were expressed as mean  $\pm$  S.E.M. Data for Relative brain weight was analysed using One-way ANOVA, followed by Post hoc LSD. While body weight was analysed using Student dependent T-test. Values were considered significant at  $P < 0.05$ .

## 3. RESULTS

### 3.1 Physical Observation

At the beginning of the experiment, all animals looked healthy. As the experiment progressed, hyper activity was observed in Group C and Group D which was exposed to the Cannabis smoke. After exposure, the exposed rats immediately fell asleep. Only the two control groups – Group A and Group B showed no clinical signs.

Table 1 revealed a non-significant ( $p < 0.05$ ) increase in the body weight in groups A, B, C,

and D when initial weight was compared to final weight.

Data was analyzed using ANOVA followed by post Hoc LSD and values were significant at  $p < 0.05$ . Result showed a non-significant ( $p > 0.05$ ) increase in groups B and D and non-significant decrease ( $p > 0.05$ ) in group C compared to group A.

Table 3 Result revealed a significant decrease in the time to locate the escape position using the Water Morris Maze test in group A; groups B and C had a non-significant decrease ( $p < 0.05$ ), and group D showed significant ( $p < 0.05$ ) decrease when the initial was compared to final.

#### 4. DISCUSSION

Cannabis contains varying amounts of the potential therapeutic compound cannabidiol (CBD), which may help quell anxiety. The medical field has seen a ramp in the attention cannabis has gotten over the past decade. The present study investigated the short term influence of cannabis smoke on the cognitive and histological parameters of the hippocampus (Figs. 1-4).

Different studies have reported inconsistent effects of cannabis use on body weight. For instance, Huang et al., [21] reported an increased risk of obesity, whereas other studies

suggest it doesn't impact weight [22]. Remarkably, cannabis has also been shown to produce weight loss [23]. The current study shows that exposure to cannabis smoke in the short term caused a non-significant ( $p > 0.05$ ) increase in the body weight in groups A, B, C, and D when the initial weight compared to the final weight. The findings from this data suggest that the short term effect of cannabis smoke on body weight is not robust/ non-significant. The result also shows a non-significant ( $p > 0.05$ ) increase in the brain weight of groups B and D and a non-significant decrease ( $p > 0.05$ ) in group C compared to group A.

The neurobehavioural study revealed a significant decrease in the time to locate the escape position using the Morris Water Maze test in group A; groups B and C had a non-significant decrease ( $P > 0.05$ ). However, in group D, which was exposed to higher doses of cannabis smoke, there was a significant decrease in the time it took for the Wister rats to locate the escape point compared to the INITIAL time it took before exposure ( $p < 0.05$ ). Indeed, exposure to cannabis can have a lasting effect on cognitive development (Gunnar et al., 2007). Although the data from this study shows that cannabis exposure impairs cognition, Higuera–Matas et al., [24] conflicts this study with findings that show that cannabis has the potential to facilitate some forms of cognitive function.

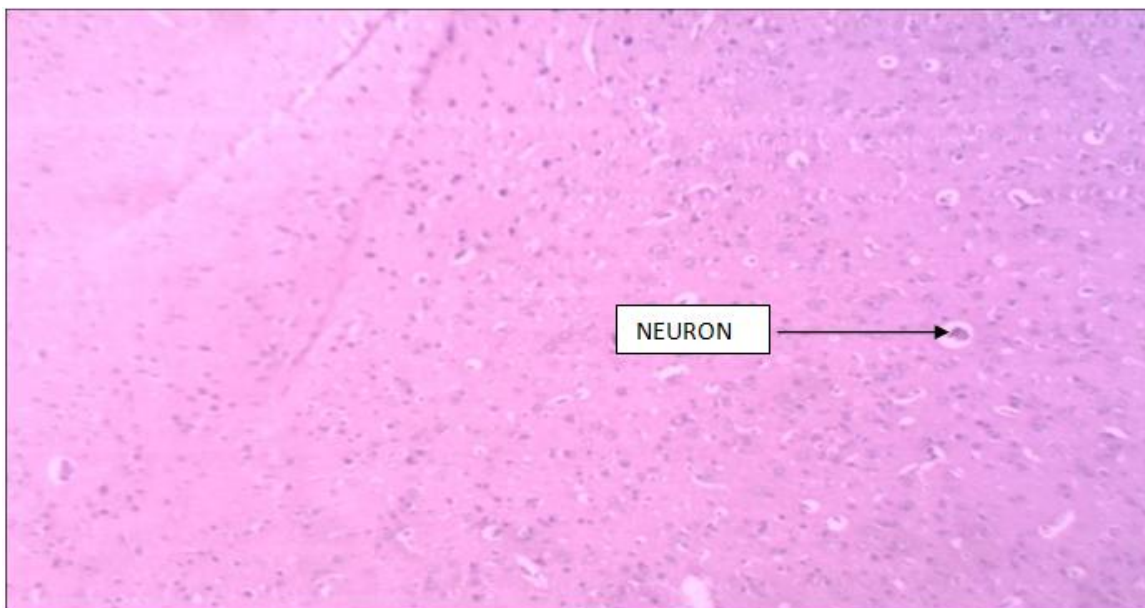


Fig. 1. Photomicrograph of rat hippocampal tissue from group a with normal structure (H&E) x100

**Table 1. Effect of Cannabis inhalation on the body weight of Wistar rats**

Group	Intialweight	Final weight	P-value	Percentage
A	120±14.35	127.4±14.93	0.223	6.167%
B	117±11.01	125±13.0	0.162	6.4%
C	126.0±14.88	129.4±5.27	0.321	2.70%
D	131.8±13.29	137.2±15.15	0.282	4.10%

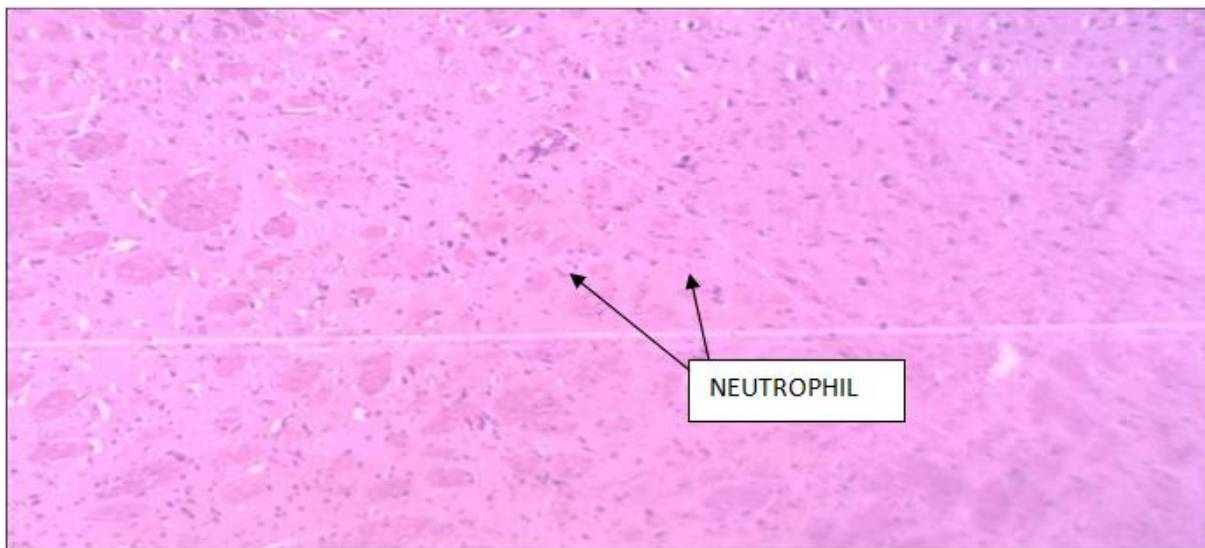
Data was analyzed using t-test and values were considered significant at  $p < 0.05$

**Table 2. Effect of cannabis inhalation on the relative brain weight in wistar rats**

Relative brain weight (g)	Mean	±SEM	P-value
Group A (control)	1.06	±0.16	
Group B (10 mins in the inhalation chamber)	1.07	±0.07	0.961
Group C (1g of cannabis for 10 mins)	0.58	±0.28	0.103
Group D (2g of cannabis for 15 mins)	1.27	±0.14	0.445

**Table 3. Effect of cannabis inhalation on morris water maze test**

Group	Initial (s)	Final(s)	P-value
A	44.6±36.13	4.0±2.34	0.018*
B	13±7.48	16.2±18.40	0.364
C	21.0±21.79	39.0±32.46	0.166
D	13.0±11.68	37.8±26.97	0.047*

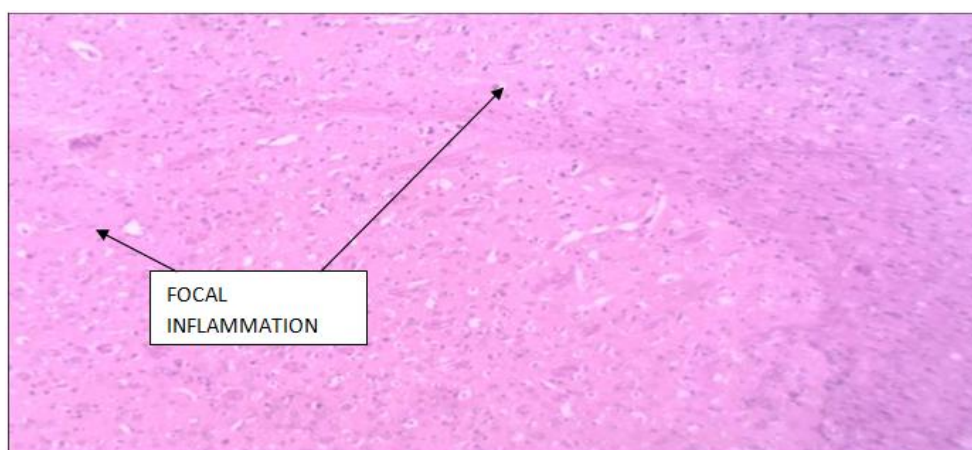


**Fig. 2. Photomicrograph of rat hippocampus in group b with neutrophil (H&E)X100**

The histological findings of this study reveal a normal formation of neurons in groups A and B. In experimental groups C and D, which was exposed to 1g and 2g of cannabis smoke, there were mild focal inflammations and alterations in the normal cell histology of the hippocampus. This finding is in line with the work of Lawston et al., [25] who reported distinct hippocampal neuron alterations, cellular infiltration, and reductions in pyramidal cell density following

cannabis smoke inhalation. Group B was placed in the inhalation chamber with a limited oxygen supply for 15 minutes to compare the findings to other experimental groups. The results show no structural and neurobehavioural difference between group B and control group A on short term exposure. From this study, the data infers that limited oxygen on short term exposure has no significant histological or neurobehavioral significance on Wister rats.





**Fig. 3. Photomicrograph of rat hippocampus from group c with focal inflammation (H&E) X100**



**Fig. 4. Photomicrograph of rat hippocampus in group d showing focal inflammation (H&E) X100**

## 5. CONCLUSION

The findings in this study reveal that cannabis smoke does not affect Wistar rats' body weight and brain weight on short-term exposure. However, it shows mild focal inflammations and alterations in the normal neuronal architecture of the hippocampal cells. On the other hand, the study revealed a decreased learning pattern and memory retrieval time after short-term cannabis smoke exposure in Morris water maze test.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Ethical approval was obtained from the ethical committee, Faculty of Basic Medical Science, College of Health Science, Nnamdi Azikiwe University, Nnewi campus. Animal handling and

treatments conform to guidelines of the National Institute of Health (NIH, 1985) for laboratory animal care and use. Assigned ethical approval number is CHS/FBMS/2021/00062.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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